REMARKS

Applicant respectfully requests reconsideration of the present application in view of the foregoing amendments and in view of the reasons that follow.

Claims 1-3 are currently being amended.

This amendment adds, changes and/or deletes claims in this application. A detailed listing of all claims that are, or were, in the application, irrespective of whether the claim(s) remain under examination in the application, is presented, with an appropriate defined status identifier.

After amending the claims as set forth above, claims 1-33 are now pending in this application.

Rejections under 35 U.S.C. § 112, paragraph 2

The Examiner rejected claims 1 and 2 under 35 U.S.C. § 112, paragraph 2 alleging that the claims were vague and confusing due to use of the phrase "and that is isotype/subtype", asserting that it was unclear whether "that" refers to the analyte or the immunoglobulin. While Applicant does not agree that claims 1 and 2 were unclear, in order to facilitate prosecution, those claims were amended above. Applicant submits that the amendments obviate the Examiner's objection.

The Examiner also rejected claim 3 under 35 U.S.C. § 112, paragraph 2, indicating that the word "then" should be replaced by "than". Claim 3 is amended above as suggested by the Examiner, thereby obviating the objection.

In view of the amendments to claims 1, 2, and 3, Applicant requests that the Examiner withdraw these rejections.

Rejections under 35 U.S.C. § 103

The Examiner rejected claims 1, 2-5, 10, 14-18, 25-26, and 28-30 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Karkmann et al (J. Immu. Methods. 1999) in view of Todisco et al. (Blood (2000) 95:535-42); rejected claims 1-2, 5, 11-19, and 23-33 as allegedly being unpatentable over Lollini et al. (Immunological Blackboard, 1998) in view of Todisco et al.; and rejected claims 6-9 and 20-22 as allegedly being unpatentable over Karkman et al. or Lollini et al. Karkmann et al. and Lollini et al. were discussed in a previous Amendment. With respect to Todisco et al. the Examiner asserted that Todisco et al. teach applying isotype-matched "nonreactive antibody" (immunoglobulin) as a negative control, i.e., to reduce non specific binding in flow cytometry to detect antigens from patients' bone marrow cells. Applicant respectfully traverses these rejections.

Because the reasons for the rejections over Karkmann in view of Todisco, and over Lollini in view of Todisco involve a common reference (Todisco) and similar reasoning, those rejections are discussed together below.

Except for the Todisco et al. reference, the other references cited by the Examiner have been previously discussed in response to claim rejections by the Examiner. In particular, Karkman et al. and Lollini et al. were discussed in the Amendment filed October 16, 2002 and in the Declaration of Dr. David R. Kaplan filed therewith. In response to those filings, the Examiner specifically withdrew the rejection of claims 3-4, 6-9, and 20-22 under 35 U.S.C. 103 over Karkmann et al. or Lollini et al. Despite that consideration and the previous withdrawal of those rejections, the present Examiner has now reintroduced essentially the same rejection.

In view of the prior discussion of the Karkmann et al. and Lollini et al. references, Applicant respectfully requests that the Examiner review and consider the Amendment and Declaration submitted October 16, 2002 for discussion of differences between those references and the present invention and reasons why those references do not make the present invention obvious. Along with other points, that prior discussion and Declaration pointed out that a skilled artisan would not understand either Karkmann or Lollini as disclosing the present claimed invention because the described conditions do not provide specific staining or a 10-fold

enhancement of signal in comparison to standard flow cytometry methods when isotype/subtype matched nonspecific immunoglobulin is used as a negative control. As pointed out in the 10/16/02 Amendment and Declaration, while the present invention provides at least a 10-fold enhancement, those references do not.

In the new rejections, the Examiner also cited Todisco et al., Blood (2000) 95: 535-542. It appears that the Examiner has misunderstood the results presented in that reference. The examiner asserts (Office Action mailed 07/1/2003, pages 3-4) that Todisco teaches "applying isotype-matched 'nonreactive antibody' (immunoglobulin) as a negative control, i.e. to reduce nonspecific binding, in flow cytometry to detect antigens from patients' bone marrow cells (... page 537, Left column)". Contrary to the Examiner's assertion, Todisco et al. did not use control Ig as a control for staining but instead used control Ig as a control for anti-CD38 treatment of the cells in culture for 7 days prior to staining with antibodies specific for CD34 and myeloperoxidase (see Figure 2). The reference indicates that anti-CD38 treatment inhibits the number of cells recovered from the cultures compared to nonreactive control Ig treatment. Consequently, there are more cells recovered with the control treatment than with the anti-CD38 treatment. The Examiner has confused the effect noted in Todisco et al. by stating that the signal (i.e., staining) Karkmann et al. obtained "would be even greater when incorporating with the standard negative control method as taught by Todisco et al.".

Likewise the Examiner uses faulty argument in asserting that "using isotype/subtype antibodies as negative control is a common knowledge in the art to reduce nonspecific binding". To the contrary, negative controls such as isotype/subtype matched immunoglobulin do not reduce nonspecific binding at all. A negative control simply allows a person to determine how much of the signal obtained can be attributed to specific binding and how much should be attributed to nonspecific binding. It does not affect nonspecific binding.

Thus, as previously discussed in the Amendment and Declaration of Dr. David R. Kaplan filed 10/16/2002, Karkmann et al. and Lollini did not use appropriate negative controls as understood by those skilled in the art. Indeed, as discussed in the Declaration of Dr. David R.

Kaplan, the effect of using appropriate negative controls would be to decrease the specific fluorescence obtained so that Lollini's claim of 10-15 fold enhancement would be decreased by 2-4 fold giving an actual 2-7.5 fold specific fluorescent signal. (See, Declaration of Dr. David R. Kaplan paragraphs 7 & 8.)

To briefly summarize, neither Karkmann et al. nor Lollini et al. describe any method that would provide at least 10-fold signal enhancement over standard flow cytometry methods. Furthermore, contrary to the Examiner's assertions, Todisco et al. does not describe or suggest using as a negative control an immunoglobulin that does not specifically bind to the analyte and is isotype/subtype matched to the analyte-specific immunoglobulin. Furthermore, one or ordinary skill in the art would understand that use of such non-specifically binding isotype/subtype matched immunoglobulin would not have the effect on binding asserted by the Examiner. Therefore, also contrary to the Examiner's assertion, the addition of Todisco et al. to either of Karkmann et al. or Lollini et al. would not lead the present methods as claimed that provide at least a 10-fold signal enhancement.

In response to the rejection of claims 6-9 and 20-22 over Karkmann et al. or Lollini et al., it appears that the Examiner has overlooked the discussion of this rejection in a previously filed Amendment filed 10/16/02) submitted to the previous Examiner. Applicant respectfully requests that the Examiner review and consider the discussion of the same rejection on page 8 last paragraph and page 9 first paragraph of the 10/16/02 Amendment. In particular, Applicant requests that the Examiner note that before optimization of a result effective variable can be held to involve no more than routine experimentation, the cited art must recognize the variable as a "result effective variable." As further pointed out in that Amendment, nothing of record indicates that the addition of high percentages of serum in a tyramide deposition method is a "result effective variable." Therefore, the Examiner has not made out a *prima facie* case of obviousness of claims 6-9 and 20-22.

Further supporting the non-obviousness of claims 6-9 and 20-22, and contradicting the Examiner assertion that "discovering an optimum value of a result effective variable involves only routine skill in the art" in relation to using 50% or 95% fetal bovine serum as a diluent in the staining process is the past practices of those of skill in the art using serum as a diluent in staining. However, optimizing a variable is only reasonable within a reasonable range. In the context of the present invention, most diluents contain 1% or 2% fetal bovine serum in order to prevent nonspecific binding of an antibody. In 30 years of performing this type of experiment and associated knowledge of work in the field, the Applicant knows of only one case where even as much as 10% fetal bovine serum was used, and no cases where greater than 10% was used. Therefore, the use of 50% (claims 6 & 20) or 95% (claims 8 & 22) fetal bovine serum represents a totally novel composition for the diluent that is not within the range of compositions known by those skilled in the art. As a result, not only does the cited art fail to recognize serum concentration as a result effective variable, the concentrations normally used by others would not provide any suggestion to use or even test the dramatically higher concentrations specified in claims 6-9 and 20-22.

In view of the discussion above and in the Amendment and Declaration of Dr. David R. Kaplan filed 10/16/02, Applicant respectfully submits that the Examiner has not made out a *prima facie* case of obviousness, or in the alternative, if any *prima facie* case of obviousness has been made, it has been rebutted. Therefore, Applicant requests that the Examiner reconsider and withdraw the rejections under 35 U.S.C. § 103.

Applicant believes that the present application is now in condition for allowance. Favorable reconsideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

The Commissioner is hereby authorized to charge any additional fees which may be required regarding this application under 37 C.F.R. §§ 1.16-1.17, or credit any overpayment, to Deposit Account No. 50-0872. Should no proper payment be enclosed herewith, as by a check being in the wrong amount, unsigned, post-dated, otherwise improper or informal or even entirely missing, the Commissioner is authorized to charge the unpaid amount to Deposit Account No. 50-0872. If any extensions of time are needed for timely acceptance of papers submitted herewith, Applicant hereby petitions for such extension under 37 C.F.R. §1.136 and authorizes payment of any such extensions fees to Deposit Account No. 50-0872.

Respectfully submitted,

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